HIGH-SPEED LIQUID CHROMATOGRAPHY

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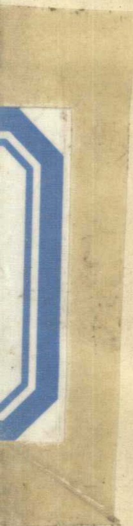

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PREFACE

Recent advances in liquid chromatography indicate that this old technique is about to enter a new era. Research work carried out during the past few years has provided a wealth of information on the principles, instrumentation, and application of high-speed liquid chromatography, so that a summary may be of value to the liquid chromatographer in most branches of chemistry, and in the fields of medicine, ecology, and pollution. The aim of this monograph is to summarize the state of the art of liquid chromatography, and in doing so we hope to provide investigators in this field with a useful tool.

Although other terms, such as high-pressure, modern, or high-performance liquid chromatography have been used by some investigators, we have chosen the title "High-Speed Liquid Chromatography" because high speed is a functional requirement, whereas high pressure, high sensitivity, and high performance only concern instrumentation.

Our own experiments as well as literature data indicate that the current status of high-speed liquid chromatography has reached a level comparable to that of gas chromatography in the early 1960s, prior to the spectacular advances in this field.

Therefore, we predict a similar dramatic increase in the literature on high-speed liquid chromatography over the next two or three years, during which time the number of papers dealing with high-speed liquid chromatography could increase sixfold.

We wish to thank all our colleagues who have contributed to the preparation of this book, and also Dr. C. Horvath for his continuous interest and valuable help in our work.

Peter M. Rajcsanyi
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INTRODUCTION

Although classical column liquid chromatography has been an effective separation method since the beginning of this century, it is still characterized by low column efficiencies and long separation times [1]. The linear velocity of the mobile phase in the column does not exceed 10^{-2} to 10^{-3} cm/sec. With respect to the time required for chromatographic separation, gas chromatography (GC) had certain advantages over liquid chromatography (LC), at least in its early phase of development; the GC method, however, is limited to volatile compounds. Moreover, thin-layer and paper chromatography, despite some advantages, exhibit other limitations. In view of this, an attempt to improve separation by LC seemed to be justified. It was obvious that improving the LC technique to a point where its efficiency and separation time could compete successfully with those of GC would permit application of LC to the analysis of a wide range of compounds. High-speed liquid chromatography (HSLC), including liquid-liquid (LLC) chromatography, gel-permeation (GPC), ion-exchange (IEC), and liquid-solid (LSC) chromatography, was expected to enable chromatographic separation of compounds with molecular weights ranging from 2×10^2 to 10^8.

In this book, the discussion of HSLC will be centered on LL partition chromatography and LS adsorption chromatography. The theory of GPC and IEC will not be treated [2-4]. We shall place emphasis on the following three major steps of development: an appropriate theoretical basis, a satisfactory instrumentation system, and experience in application of the system, all of which are important for reducing the analysis time and improving separation efficiency.

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Chapter 1

THEORETICAL BASIS FOR HSLC

The basis of chromatographic separation is the distribution (or partition) of sample components between two phases which are immiscible. Thus separation depends on both the mobile or moving phase and the stationary phase. Interactions between the molecules of the two phases are negligible in GC, but play a very important role in LC. These interactions of the liquid chromatographic system determine the degree of sorption of particular substances and also the effectiveness or selectivity of the separations. (See Fig. 1.1.) As is well known from the general theory of chromatography the retention and dispersion of a solute are determined by both thermodynamic and kinetic parameters [5-8]. It is very advantageous that these two groups of parameters are almost independent of each other and may be separately optimized. On the other hand, it requires full knowledge of the factors affecting peak separation, which are, as follows from the foregoing, rather numerous. About ten years ago the theory developed for GC was believed to be easily applied to LC [9-12]. Although this was realized as early as 1960 by Hamilton et al. [13], by Karr et al. [14] in 1963, and confirmed by Piel [15] in 1966, the rules for high-speed liquid

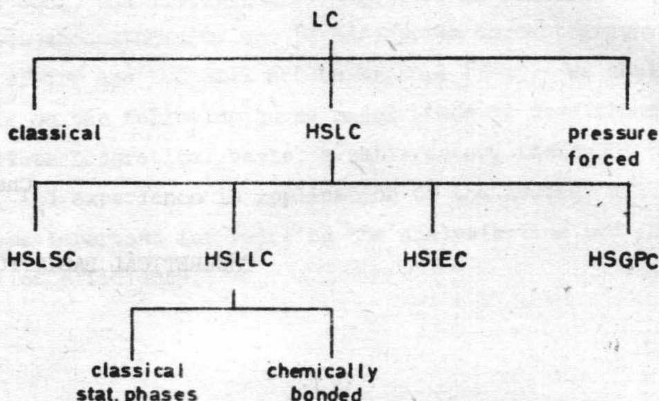

FIG. 1.1 Branches of liquid chromatography.

chromatographic separation are still much less known than for GC. The explanation of this fact lies in the complexity of HSLC.

1.1 THERMODYNAMICS OF LLC

The sample components in a column are continuously partitioned between the mobile and stationary phases in constant ratios, named distribution or partition coefficient [16,17]:

$$K = \frac{\text{solute concentration in stationary phase}}{\text{solute concentration in mobile phase}} \qquad (1)$$

The solute distribution coefficient must be constant during the migration of the solute down the column, that is, a linear partition isotherm is preferred. (At this point several assumptions must be taken into consideration according to Locke and Martire [16].)

For LLC, linear isotherms may be expected up to about 1% volume fraction; for LSC, linear isotherms are rare and the problem is more difficult. If V_s is the volume of stationary

phase in the column and V_m is the interstitial volume of the column, the retention volume of a component, for which K differs from zero, is defined as

$$V_R = V_m + KV_s \qquad (2)$$

Considering solute residence times due to solute-liquid phase interactions, the net retention volume of a component can be defined:

$$V_N = V_R - V_m = KV_s \qquad (3)$$

and from (3) we obtain an equation for the specific retention volume

$$V_g = \frac{V_N}{w_s} = \frac{K}{\rho_s} \qquad (4)$$

where w_s is the weight of stationary phase in the column and ρ_s its density.

A new important parameter, the partition ratio or capacity factor, can be obtained by multiplying the partition coefficient by the phase ratio $\beta = V_s/V_m$, i.e., the volume ratio of the two phases

$$k = K\beta = K\frac{V_s}{V_m} = \frac{V_N}{V_m} \qquad (5)$$

The partition ratio has theoretically a simple physicochemical meaning: it is the number of component molecules in the moving phase per component molecules in the fixed phase. It means practically the time an average solute molecule spends in the mobile phase relative to that spent in the stationary phase, and can be determined directly from the chromatogram. Taking the condition of equilibrium in LLC at pressure P and temperature T

$$\mu^s(T,P) = \mu^m(T,P) \tag{6}$$

where μ is the solute chemical potential, s and m indicate the stationary and mobile phases, respectively, we have for the specific retention volume

$$\ln V_g = \ln \frac{\gamma^{m,\infty}(T,P = 1)M_m}{\gamma^{s,\infty}(T,P = 1)M_s\rho_m(T)} + \frac{\bar{P} - 1}{RT}(v^m - v^s) \tag{7}$$

where $\gamma^{m,\infty}$ and $\gamma^{s,\infty}$ are the solute activity coefficients at infinite dilution, M_m and M_s are the molecular weights of the phases and $\rho_m(T)$ is the mobile phase density at temperature T, $\bar{P}$ is the mean column pressure, v^m and v^s are the solute molar volumes in the two phases. This equation allows us to predict partition properties, partition coefficients and retention volumes a priori for systems in which the activity coefficients are known [18-20]. In the dynamic determination of partition coefficients, developed by Huber and co-workers [19,21], a linear relationship is assumed between the retention time and K:

$$t_R = t_0(1 + \beta K) \tag{8}$$

where t_0 is the average residence time of the mobile phase. The unknown partition coefficient of a compound can be calculated by means of Eq. (8) from its retention time and the retention times of other compounds with known partition coefficients measured under the same conditions.

The main sources of error of this method may be adsorption effects and nonlinearity of the distribution isotherm. Partition coefficients of 26 steroids, 6 alkyl benzenes, 5 nitro derivatives of benzene, and 13 pesticides were determined by this method [21]. The apparent limiting activity coefficients were determined by measuring the specific retention volume values of

naphthalene, paraffins, olefins, and aromatic hydrocarbons in the range of C_4 to C_{14} in an LLC system, where acetonitrile served as the mobile phase and squalane as the stationary phase [22]. From these values, the excess partial molar free energies, enthalpies, and entropies of mixing were calculated. In the case of two components (1 and 2) in a solute the relative retention

$$\alpha = \left(\frac{\gamma^{m,\infty}}{\gamma^{s,\infty}}\right)_1 \left(\frac{\gamma^{s,\infty}}{\gamma^{m,\infty}}\right)_2 \qquad (9)$$

will depend only on the differences in the solution behavior of the components in the two phases. That is, the relative retention is really a measure of the thermodynamic differences of component distribution, or the difference in free energies of distribution for two components. In LLC one can, therefore, improve separation by changing mainly the nature of the mobile phase; not only that of the stationary phase as in GC. (The improvement of separation in LLC can also be carried out by changing the temperature.) Thus, the use of gradient elution constitutes a bridge between theory and practice.

All in all, from the thermodynamic basis of LLC it is clear that resolution of two adjacent bands can be improved by changing the separation factor or relative retention (α) and the partition ratio (k) [23-27]. In HSLC the role of the capacity factor in resolution cannot be neglected, since the low volume ratio of stationary to mobile phase involves a low k value. These low k values are indeed desired for high speed, as discussed later, but for resolution high k values are preferable. Therefore a compromise must obviously be made, that is, there is an optimal capacity factor value, but with a single solvent k is never optimum for all components of the sample. Thermodynamically, the value of the capacity factor depends on the solvent strength, and strong solvents give smaller k values. Horvath and Lipsky

[28] noted that the peak capacity can be significantly improved by use of gradient elution. In addition to this, we should note that the factors--capacity ratio and relative retention--affecting separation are not independent of each other to the effect that, although k was optimized for every pair of adjacent bands, either a favorable change in the separation factor, i.e., selectivity, or a further increase in column efficiency may be necessary. Alteration in the α value, while keeping k approximately constant, can be achieved by varying the solvent composition. For this purpose mainly practical approaches are at our disposal. Therefore the choice of stationary and mobile phases is one of the weakest points in the development of HSLC [29].

Since the partition coefficient is an equilibrium constant thermodynamically involving Gibbs' chemical potential and activity coefficients, it is dependent on the identity of the solute, the mobile and stationary phases and interactions, as well as upon the temperature [30]. Therefore, both athermal (size) and thermal (energy) factors contribute to the relative retention, i.e., selectivity [31]. Luckhurst, Martire, and Locke developed the above new approach, in order to consider the thermodynamic basis of selectivity in LLC [31,32]. (Their theory is inapplicable to systems where solvent-solvent interaction is stronger than interactions between solute and solvent.) Eon et al. [33] showed that the interfacial tension between the mobile and stationary phases reflects the partition properties of the two phases, and this could be taken as a criterion of choice of the systems to be used in HSLC.

1.2 THERMODYNAMICS OF LSC

In LSC the distribution coefficient of a component between mobile and stationary phases depends on interaction forces; mainly on dispersion forces (nonpolar) and on hydrogen bonding (polar)

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